

Laboratory Evaluation of the Novel Naturally Derived Compound Spinosad against *Ceratitis capitata*

Angeles Adán, Pedro Del Estal, Flor Budia, Manuel González & Elisa Viñuela*

Unidad de Protección de Cultivos, ETSI Agrónomos, E-28040-Madrid, Spain

(Received 20 November 1995; revised version received 29 April 1996; accepted 8 July 1996)

Abstract: Laboratory studies were conducted to determine the effect of the naturally derived compound spinosad on *Ceratitis capitata* Wied. (Diptera, Tephritidae). The organophosphate fenthion was used as a standard. Direct dose-dependent mortality and reduced fecundity were observed in oral treatment of adults with spinosad. The LC_{90} values 14 h and seven days after treatment were 19.50 and 0.49 mg litre⁻¹ respectively. Fenthion was less active (the LC_{50} eight days after treatment was 1.17 mg litre⁻¹) and did not affect the fecundity of the fly. Adults were also very susceptible to spinosad and fenthion *via* residual contact. For spinosad, 100% mortality was recorded 48 h after treatment for a dose of 10 mg litre⁻¹. Spinosad was more effective than fenthion in suppressing larval development when neonate larvae were reared on treated diet supplemented with a range of concentrations from 0.02 to 0.83 mg kg⁻¹ diet. Last-instar larvae were much less susceptible to spinosad or fenthion when exposed *via* dipping or when they pupated in treated medium and both products had similar performance. A lack of ovicidal activity was observed in direct egg-treatments with spinosad but significant reductions from 1 mg litre⁻¹ onwards were recorded for fenthion.

Key words: naturally derived compound, spinosad, fenthion, *Ceratitis capitata*

1 INTRODUCTION

The spinosyns, recently discovered by DowElanco, represent a novel class of macrocyclic lactones produced by the soil actinomycete *Saccharopolyspora spinosa*.¹ These products are a complex of several natural metabolites and have shown good biological activity against insects of different orders. The code compound XDE-105 or spinosad (proposed common name), is a mixture of spinosyns A and D, the two most active natural factors identified up to date and it will be the first representative of this group to be commercialized.¹

Spinosad acts both as contact and stomach poison, and it is particularly effective against Lepidoptera and Diptera, although some activity against other insect pests of different orders has also been reported.^{1–3} Furthermore, this new insect control agent has very low

mammalian toxicity and it is considered safe for many non-target organisms.^{1–4} The compound acts on the insect nervous system in a novel manner, but the actual molecular mode of action is still unknown.¹

Ceratitis capitata Wied. is an economically important widespread pest of subtropical and deciduous fruits which is under quarantine regulations in many countries.⁵ All over the Mediterranean regions, it is a very serious pest because more than 250 types of fruits grown commercially are susceptible hosts and because the number of generations per year is very high.^{5,6} In Spain, damage caused to citrus production has led to mandatory control measures in this crop since 1955 in order to avoid export difficulties. Due to the biological characteristics of this fly (immature development takes place inside the fruit and pupation takes place in the soil), treatments are focused upon adults and the currently used pesticides are broad-spectrum organophosphates or pyrethroids. Other developmental stages

* To whom correspondence should be addressed.

can, however, be targeted by the insecticides in special situations. Localized soil treatments against mature larvae or young pupae have given good results as complementary measures in eradication programmes in the USA.⁷ These treatments can also be interesting in small orchards because their effect may last longer than that of sprays.⁸ On the other hand, in post-harvest disinfection, penetrating insecticides are used to eliminate eggs and neonate larvae in fruits.⁹

In order to decrease the environmental impact of treatments, there is a need to find more benign alternatives for the control of this fly. Biorational control agents, based on natural products are potential candidates.

The objective of the current laboratory study was to assess the susceptibility of *C. capitata* adults to spinosad when they were exposed to the insecticide *via* different methods. Effects on other developmental stages were also investigated. The organophosphate fenthion was used as standard because it is one of the insecticides currently used in *C. capitata* control programmes in Spain.

2 EXPERIMENTAL METHODS

2.1 Chemicals

The test chemicals were a 480 g litre⁻¹ suspension concentrate formulation of spinosad provided by Dow-Elanco Ibérica Co. (Madrid) and a 500 g litre⁻¹ emulsifiable concentrate formulation of fenthion (Lebaycid, Bayer Hispania comercial, Barcelona). Fresh dispersions of both compounds in distilled water were prepared prior to the assays. The structure of spinosad is given in Fig. 1.

2.2 Insects

Flies used in these tests came from a mass-reared stock maintained in our laboratory with no history of insecticide exposure. Flies were reared at a temperature of 25 (±2)°C, 75 (±5)% RH. and 16 : 8 h light : dark photo-

period.^{10,11} A mixture of sucrose and enzymatic autolyzed brewer's yeast ((4 + 1) by weight) was used as adult food.

Each experiment consisted of four replicates per dose level and for the control and they were performed twice, under the same conditions as described for rearing the insects. Control groups were supplied with distilled water. Carbon dioxide anaesthesia was used to facilitate the handling of adults. This anaesthetic is harmless to *C. capitata*.¹²

2.3 Oral bioassay in adults

Groups of five newly emerged pairs were placed in sealed plastic boxes with a hole on the upper side for ventilation, and a lateral oviposition device provided with a moisture source to attract females to oviposit.¹³

Spinosad and fenthion were offered *ad libitum* to adults in the drinking water with small glass vials covered with 'Parafilm®' and a piece of 'Spontex®' wiper. Ten different concentrations ranging from 0.1 to 20 mg of active ingredient (AI) litre⁻¹ were investigated. Food was supplied in small containers. Neither food nor insecticide solution was replaced during the assay.

Daily mortality was monitored until flies were 10 days old, because in this period natural adult mortality is still low. The cumulative number of eggs per female during a five-day period from the starting of the oviposition, at the two lowest doses, was used to study fecundity. For the rest of doses, mortality in spinosad-treated units was too high to allow this parameter to be studied.

To detect possible feeding inhibition effects, feeding behaviour of spinosad-treated insects was checked every two hours the first day of the assay.

2.4 Residual contact bioassay with adults

Groups of five newly emerged pairs, were kept in glass dismountable cages consisting of two glass plates and a round brass frame, and provided with food and water.¹¹ Plates were treated under a Potter precision spray

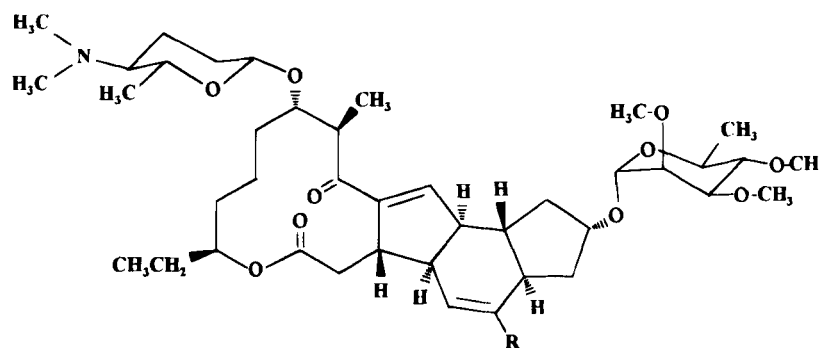


Fig. 1. Structural formula of spinosad.

tower,¹⁴ with a standard deposit of 1.5 (± 0.04) mg cm⁻² (1 ml; 50 kPa). Three concentrations were tested: 1, 10 and 100 mg AI litre⁻¹. As soon as the plates were dry, they were mounted with two bolts and adults were introduced into the cages. Cages were then transferred to the climatic chamber and connected to forced ventilation. Daily mortality was assessed as previously described.

2.5 Egg bioassay

To evaluate the ovicidal effect of both chemicals, samples of 50 eggs less than 24 hours old collected from seven- to eight-day-old flies, were used. Eggs were placed on black filter paper treated with 1 ml of the different insecticide concentrations, in sealed plastic cages (9 cm diam, 3 cm high). Four to six concentrations ranging from 1 to 1000 mg AI litre⁻¹ were used in these studies. Egg hatching was evaluated 48 h later.

2.6 Larval exposure by ingestion

Groups of 50 newly hatched larvae were reared in the presence of spinosad- or fenthion-treatment diet in sealed plastic boxes (9 cm diam, 3 cm high) containing 50 g of the larval rearing medium. Insecticide solutions (five to six different concentrations from 0.019 to 0.83 mg AI kg⁻¹ diet) were mixed with the diet (5 ml per unit) before adding the insects. To obtain larvae of uniform age, groups of 100 eggs were placed on black filter paper in the sealed plastic cages previously described. Two days later, emerged larvae, randomly chosen, were used for the assays.

Larval and pupal mortality were estimated from the number of total pupae obtained and emerging flies, respectively. In the laboratory, fully grown larvae of this fly jump off the diet to pupate in a drier substratum. Thus larval mobility could also be scored by counting the number of popped pupae compared to pupae.

2.7 Larval exposure *via* dipping

Groups of 10 seven-day-old popped (mature 3rd-instar) larvae were dipped in four different concentrations of each insecticide ranging from 10 to 10 000 mg AI litre⁻¹ for 10 min. Insects were dried for five minutes on filter paper and allowed to pupate normally.

Larval and pupal mortality were scored as described in the exposure *via* ingestion.

2.8 Treatment of the medium of pupation

Groups of 10 seven-day-old popped larvae were allowed to pupate in plastic boxes (12 cm diam, 5.5 cm high) filled with 48 g of vermiculite treated with insecticide. In

every unit, 12 ml of 5 different insecticide solutions (from 10 to 10 000 mg AI litre⁻¹) were added and dried at room temperature and natural daylight conditions (approximately 12 h light photoperiod) for 48 h, before adding the insects. Three days later, completely hardened pupae were separated and put in ventilated plastic cages (similar to those described in adult treatments) until adult emergence. Larval and pupal mortality were evaluated as previously described.

2.9 Statistical analysis

One-way analysis of variance (ANOVA) and the LSD multiple range test were performed on the data to determine significant ($P = 0.05$) dose differences.¹⁵ A Bonferroni test was applied to the means when the test F from ANOVA was non-significant. Data were transformed where appropriate to $\arcsin \sqrt{x}$, and are presented as means \pm standard errors.

In some cases, mortality data could be subjected to POLO-PC,¹⁶ to estimate probit regressions. Lethal concentrations (LC) were calculated in mg AI litre⁻¹ or mg AI kg⁻¹ diet, and the 95% fiducial limits (FL) were determined for the LC₅₀ and LC₉₀ values. At least five concentrations were used for determination of LC values. The criterion of overlapping fiducial limits of relative potencies of lines was used to establish whether lines were significantly different or not ($P = 0.05$).

3 RESULTS AND DISCUSSION

Very few data are available on the insecticidal activity of spinosad. The compound affects both adults and larvae of species belonging to different insect orders and it has a promising insecticidal activity *via* different exposure methods.¹⁻³

3.1 Adult bioassays

The adults of *C. capitata* were highly susceptible to spinosad when applied both *via* drinking water or residual contact. Affected insects were progressively immobilized, showed uncontrolled movements and were dead shortly afterwards.

On feeding assays, spinosad had a much higher toxicity to the fly than fenthion and its efficacy was dependent on the concentration and on the exposure time to the insecticide. Effect of selected doses of both insecticides is shown in Table 1. The highest dosage of spinosad produced high mortality within one day and the lowest dosages which caused little or no early mortality also caused high mortalities with time. In contrast with these results, the highest mean mortality recorded for fenthion on day 1 was negligible and by day 4 never exceeded 65% even at the concentration of 20 mg AI litre⁻¹. By day 7, however, fenthion was highly toxic

TABLE 1
Effect of Selected Doses of Spinosad and Fenthion on *Ceratitis capitata* Adults Exposed *via* Ingestion in the Drinking Water

Doses (mg AI litre ⁻¹)	Mean mortality (%) (\pm SE) ^a					
	1 day		4 days		7 days	
	Spinosad	Fenthion	Spinosad	Fenthion	Spinosad	Fenthion
0.1	2.5 (\pm 2.5)a	0a	20.0 (\pm 10)b	0a	54.5 (\pm 9.2)c	2.5 (\pm 2.5)a
0.5	10 (\pm 4.1)ab	2.5 (\pm 2.5)a	92.5 (\pm 4.8)c	7.5 (\pm 4.8)ab	91.9 (\pm 5.2)c	30.0 (\pm 15.8)b
1.5	22.5 (\pm 7.5)b	0a	100c	5.0 (\pm 2.9)a	100c	27.5 (\pm 9.5)b
4.5	37.5 (\pm 7.5)b	0a	100c	7.5 (\pm 4.8)a	100c	57.5 (\pm 13.2)b
13	52.5 (\pm 4.8)c	0a	100d	35.0 (\pm 6.5)b	100d	97.5 (\pm 2.5)d
20	80.0 (\pm 7.1)b	2.5 (\pm 2.5)a	100c	65.0 (\pm 14.4)b	100c	100c

^a Within the same line, data followed by the same letter are not significantly different ($P = 0.05$; ANOVA and LSD mean separation.) Data transformed to $\arcsin \sqrt{x}$ and corrected for control mortality by the method of Abbott.²⁶

between 13 and 20 mg AI litre⁻¹ but even now significant differences in activity could be observed compared to spinosad for the rest of the doses.

The regression lines fitted for spinosad at different time intervals, from 14 h to seven days after treatment, are represented in Table 2. Data from day 1 to day 7 could be represented by parallel probit-log dose regression lines (χ^2 value for parallelism = 16.87; 23 df) of common slope $b = 2.61 \pm 0.17$. An eight-fold increase in potency was detected between day 1 and 7.

Spinosad had a very fast action in *C. capitata*. A relatively low LC₅₀ was obtained as early as 14 h after treatment (3.5 mg AI litre⁻¹), and the LC₅₀ recorded at 48 h, 1.0 mg AI litre⁻¹, was much lower than those reported in this fly for classical insecticides such as malathion (28.2 mg litre⁻¹), carbaryl (2.1×10^4 mg

litre⁻¹), permethrin (176.7 mg litre⁻¹) or naled (1.7 mg litre⁻¹).¹⁷ Also in our assays the LC₅₀ recorded at eight days for fenthion, 1.17 mg AI litre⁻¹ (FL = 0.59–2.09; $\chi^2 = 6.71$; 4 df; $b = 1.71 \pm 0.18$) was considerably greater (six-fold) than that obtained for spinosad at seven days, 0.18 mg AI litre⁻¹ (FL = 0.12–0.23; $\chi^2 = 1.13$; 3 df; $b = 2.92 \pm 0.57$).

Feeding inhibition effects have been reported in the fly *Liriomyza trifolii* (Burgess) after treatment with avermectins, which are also fermentation products.^{18,19} However, in our assays with spinosad, during the first 24 h after starting the test, feeding behaviour of flies in treated units was not different from that of controls.

Spinosad was also very effective and fast acting *via* residual contact (Table 3). A 100% mortality was recorded in *C. capitata* 48 h after treatment with a dose

TABLE 2
Effect of Spinosad on *Ceratitis capitata* Adults Exposed *via* Ingestion in the Drinking Water

Interval after treatment	LC ₅₀ ^a (Fiducial limits)	LC ₉₀ ^a (Fiducial limits)	Slope (\pm SE)	Relative potency ^b (Fiducial limits)
14 h	3.49 (2.61–4.61)	19.53 (13.30–33.66)	1.71 (\pm 0.19)	—
1 d	1.55 (1.14–2.53)	7.13 (3.77–34.23)	1.93 (\pm 0.44)	1a
2 d	1.0 (0.73–1.23)	2.59 (1.89–5.85)	3.09 (\pm 0.77)	1.36b (1.01–1.84)
3 d	0.62 (0.45–0.77)	1.66 (1.29–2.63)	3.03 (\pm 0.59)	2.21bc (1.66–2.97)
4 d	0.40 (0.31–0.49)	1.22 (0.94–1.82)	2.63 (\pm 0.37)	3.44cd (2.59–4.65)
5 d	0.24 (0.11–0.36)	0.87 (0.58–1.57)	2.30 (\pm 0.38)	5.33de (3.96–7.34)
7 d	0.18 (0.12–0.23)	0.49 (0.39–0.73)	2.92 (\pm 0.57)	8.16ef (5.92–11.60)

^a Doses in mg AI litre⁻¹.

^b Data followed by the same letter are not significantly different ($P = 0.05$).

TABLE 3
Toxicity of Spinosad and Fenthion by Residual Contact to Adults of *Ceratitis capitata*

Mortality (%) (\pm SE) ^a								
Insecticide	Dose (mg AI litre ⁻¹)	5 h	1 day	2 days	3 days	4 days	5 days	10 days
Spinosad	0	0a	2.5 (\pm 2.1)a	7.5 (\pm 2.1)a	10.0 (\pm 0)a	17.5 (\pm 2.1)a	17.5 (\pm 2.1)a	20.0 (\pm 3.5)a
	1	0a	2.5 (\pm 2.1)a	23.3 (\pm 11.1)b	36.7 (\pm 10)b	63.3 (\pm 2.7)b	76.7 (\pm 2.8)b	76.7 (\pm 2.8)b
	10	0a	52.5 (\pm 7.3)b	100c	—	—	—	—
	100	32.5 (\pm 6.5)b	100c	—	—	—	—	—
Fenthion	0	0a	0a	0a	0a	0a	2.5 (\pm 2.2)a	13.3 (\pm 2.8)a
	1	0a	0a	0a	0a	0a	0a	5.0 (\pm 3.6)a
	10	100b	—	—	—	—	—	—
	100	100b	—	—	—	—	—	—

^a Within each column, data followed by the same letter are not significantly different ($P = 0.05$; ANOVA and LSD mean separation).

as low as 10 mg AI litre⁻¹. At the lowest dose tested (1 mg AI litre⁻¹), a progressive increase in mortality was observed from day 1 to 4. However mortality stabilized at day 5 and did not increase with time. One factor to take into account is that photolysis is the major route of degradation of this insecticide,¹ and the glass plates with insecticide deposits were maintained under a 16-h light photoperiod during the assay. When compared to spinosad, residual toxicity of fenthion against the fly at the two highest dosages tested was initially greater, and a 100% mortality was recorded as early as 5 h after treatment. At the lowest dosage, however, a total lack of effect was obtained.

Our study showed spinosad to reduce fecundity significantly at every tested dose, when flies were fed the insecticide ($F = 23.41$; $df = 2,9$; $P = 0.0003$). The cumulative number of eggs per female during a period of five days was 24.7 (\pm 4.5) at the dose of 0.32 mg AI litre⁻¹ and 38.3 (\pm 13.6) for 0.1 mg AI litre⁻¹. Both values were significantly different from those obtained with the control (196.3 (\pm 34.4)). This is a very interesting result because reproduction of *C. capitata* was unaffected by the broad-spectrum insecticide fenthion. The cumulative number of eggs per female in fenthion-treated units during the same period 213.0 (\pm 24.4) and 224.6 (\pm 21.3) at the dose of 0.1 and 0.5 mg AI litre⁻¹ respectively) was significantly equal to that of control (172.2 (\pm 11.9)) ($F = 1.43$; $df = 2,9$; $P = 0.29$).

Avermectins also affect the reproduction of different insect species, both after exposure *via* ingestion or topical applications.²⁰ However the level of activity seems to be lower, because when avermectin B₁ was topically applied to *C. capitata* adults, only a 2.5-fold reduction was observed at 0.9 mg litre⁻¹ after 17 days.²¹

3.2 Egg bioassay

Spinosad did not exhibit any ovicidal activity against *C. capitata* even at the highest dose tested (1000 mg AI

litre⁻¹). Hatching percentages ranged between 73 and 79% and were not significantly different from those in controls (Table 4).

Similar to these results, avermectins did not produce mortality in a broad range of species by direct egg treatment.¹⁹

In contrast with spinosad, fenthion exhibited a concentration suppressive effect on eggs of *C. capitata* from very low doses and the percentage hatch was 0 from 8 mg AI litre⁻¹ onwards.

3.3 Larval bioassays

Spinosad was much more effective than fenthion in suppressing larval development when neonate larvae were reared on treated medium (Table 5). For spinosad, median larval mortality concentration, on the basis of total number of pupae, could be estimated by probit

TABLE 4
Ovicidal Activity of Spinosad and Fenthion on less than 24-h-old Eggs of *Ceratitis capitata*

Doses (mg AI litre ⁻¹)	Egg hatch (%) (\pm SE) ^a	
	Spinosad ^b	Fenthion ^c
0	78.5 (\pm 3.1)a	65.0 (\pm 2.5)a
1	79.0 (\pm 5.2)a	41.5 (\pm 5.4)b
2	—	22.5 (\pm 4.0)c
4	—	10.0 (\pm 2.1)d
6	—	1.0 (\pm 0.5)e
8	—	0
10	74.0 (\pm 3.6)a	0
100	73.0 (\pm 3.8)a	—
1000	76.0 (\pm 7.0)a	—

^a Data within columns followed by the same letter are not significantly different at the $P = 0.05$ level.

^b ANOVA and Bonferroni mean separation.

^c ANOVA and LSD mean separation.

TABLE 5

Effect of Dietary Exposure on the Percentage of Total Pupae, Adults and Popped Pupae when Newly Hatched Larvae of *Ceratitis capitata* were reared in Spinosad- or Fenthion-Treated Diet^a

Dose (mg AI kg ⁻¹)	Total pupae ^b		Adults ^c		Popped pupae ^d	
	Spinosad	Fenthion	Spinosad	Fenthion ^e	Spinosad	Fenthion
0	84.0 (±1.6)a	84.7 (±5.5)a	97.7 (±1.9)a	92.2 (±3.6)ab	72.7 (±11.4)a	89.6 (±2.8)a
0.019	30.0 (±0.0)b	84.7 (±10.4)a	100a	96.0 (±1.7)bc	63.3 (±7.1)a	81.7 (±5.3)a
0.035	13.7 (±3.6)c	—	92.6 (±10.5)a	—	41.2 (±12.9)a	—
0.065	4.0 (±2.5)d	93.3 (±3.4)a	90.0 (±7.1)a	99.1 (±0.7)bc	0b	87.0 (±2.9)a
0.12	1.3 (±0.5)d	82.0 (±5.1)a	100a	98.3 (±0.7)bc	0b	90.9 (±3.9)a
0.22	0d	83.3 (±3.4)a	—	82.0 (±4.1)a	—	78.0 (±3.2)a
0.33	—	58.0 (±5.8)b	—	100c	—	90.9 (±4.6)a
0.83	—	13.3 (±2.0)c	—	99.3 (±0.6)bc	—	84.1 (±2.2)a

^a Data followed by the same letter, within the same column, are not significantly different at the $P = 0.05$ level (ANOVA and LSD mean separation).

^b (No. of pupae/no. of larvae) × 100.

^c (No. of adults/no. of pupae) × 100.

^d (No. of popped pupae/no. of pupae) × 100.

^e Data transformed to arcsin \sqrt{x} .

analysis. The slope of the line was $b = 2.38 \pm 0.33$ ($\chi^2 = 0.52$; 3 df), and the LC₅₀ and LC₉₀ values in mg AI kg⁻¹ diet (95% fiducial limits) were 0.013 (0.009; 0.017) and 0.045 (0.038; 0.055) respectively. However, pupal development was not affected and the percentages of adult emergence both in spinosad- or fenthion-treated units were similar to those in controls and higher than 90%.

Only spinosad had an effect on larval mobility. In treated units, larvae were less active and lower percentages of popped pupae were recorded compared to con-

trols. A lowering of locomotory activity in insects, after the ingestion of some insecticides, has been occasionally reported, and among the responsible factors are alterations of the cuticle or muscle structure.^{22,23}

When effectiveness of spinosad was tested against mature third-instar larvae (=popped larvae), which is one of the vulnerable stages of fruit flies found in soil, results differed with the exposure method (Table 6). *Via* dipping, the compound did not affect larval development (numbers of pupae were similar in control and treated units), but a decrease in the percentage of adult

TABLE 6

Influence of the Application Method on the Susceptibility of Seven-day-old *Ceratitis capitata* Popped Larvae to Spinosad and Fenthion^a

Insecticide	Dose (mg AI litre ⁻¹)	Dipping		Treatment of the medium of pupation	
		Pupae ^b	Adults ^c	Pupae ^b	Adults ^c
Spinosad	0	100a	90.0 (±3.5)a	100a	100a
	10	95.0 (±4.3)a	70.0 (±15.4)ab	100a	97.5 (±2.1)a
	100	95.0 (±4.3)a	45.0 (±4.3)b	97.5 (±2.1)a	97.5 (±2.1)a
	500	—	—	77.5 (±7.4)ab	25.0 (±7.5)b
	1000	95.0 (±2.5)a	15.0 (±2.5)c	77.5 (±8.9)ab	0c
	10000	95.5 (±2.1)a	7.5 (±6.5)c	75.0 (±2.5)b	0c
Fenthion	0	100a	87.5 (±4.1)a	100a	95.0 (±2.5)a
	10	100a	92.5 (±2.2)a	100a	70.0 (±7.9)b
	100	97.5 (±2.5)a	87.5 (±4.1)a	100a	20.0 (±14.6)c
	1000	95.0 (±2.9)a	27.5 (±7.4)c	100a	0c
	10000	100a	2.5 (±2.2)c	97.5 (±2.5)a	0c

^a Data within columns followed by the same letter are not significantly different at the $P = 0.05$ level (ANOVA and LSD mean separation).

^b (No. of pupae/no. of larvae) × 100.

^c (No. of adults/no. of pupae) × 100.

emergence was scored from the dose of 100 mg AI litre⁻¹ onwards. In contrast with these results, when larvae were allowed to pupate in treated vermiculite, both larval and pupal development were affected, but only at high doses. One factor to take into account is the length of time that insects remained in contact with the insecticide: three days in the latter treatment versus ten minutes in the former one.

The efficacy of spinosad in both treatments could probably be increased using organic solvents instead of water, because solvents are very important in facilitating insecticide penetration of the cuticle. Laboratory assays performed with the insect growth inhibitor cyromazine (as 'Trigard') dissolved in water, applied to the medium of pupation, gave good results in decreasing adult emergence of *C. capitata*, but only at doses higher than 500 mg AI litre⁻¹.²⁴ However, a good effectiveness of technical cyromazine diluted in methanol, from a dose of 0.15 mg litre⁻¹ onwards, has been reported in the same fly.²⁵

The organophosphate fenthion applied either *via* dipping or in treatment of the medium of pupation had no effect on larval development of *C. capitata*, but, similar to spinosad, inhibited the eclosion of adult flies at high doses.

4 CONCLUSION

According to the results presented, spinosad is a good candidate for the control of *C. capitata* adults. The product has shown a fast-acting effect at low doses *via* ingestion and residual contact, and significant decreases in fecundity have also been observed. The oral LC₉₀, 24 h after treatment, was as low as 7.13 mg litre⁻¹, and *via* residual contact, 100% mortality was scored two days after treatment at a dose of 10 mg litre⁻¹. Although studies should be conducted to determine its performance against *C. capitata* under field conditions, spinosad seems to be effective at lower doses than the organophosphates malathion or fenthion, which are currently applied for the control of the fly (field dosages of 500–600 g AI ha⁻¹ or 40 g AI ha⁻¹ in bait spray). On the other hand, spinosad is less toxic to rats (oral LD₅₀ in females > 5000 mg kg⁻¹, tech.) than malathion (LD₅₀ = 2800 mg kg⁻¹, tech.) or fenthion (LD₅₀ = 245–615 mg kg⁻¹, tech.). Moreover, detrimental effects of spinosad on beneficial fauna appeared negligible in preliminary assays.¹⁴ Therefore some advantages of using spinosad are that this product may be safer for the environment and less hazardous to applicators than organophosphates.

Hence, this new insect control agent may be a promising component in IPM programs to control *C. capitata*, but further research is needed to ascertain totally both its toxic action on the different developmental

stages of the fly in laboratory and its effectiveness in field trials.

ACKNOWLEDGEMENTS

Dr A. Adán and M. González acknowledges the FPI grants by the Ministerio de Educación y Ciencia de España and the Comunidad de Madrid, respectively. Authors also are indebted to Dr J. L. Buendía (DowElanco Ibérica Co) for his technical assistance, and to Dr J. A. Jacas for constructive criticism.

REFERENCES

1. DowElanco, *Spinosad Technical Guide*, 1994, 24 pp.
2. Busacca, J. D., Barber, D. T., Dintenfuss, L. P., Ksander, T. F., Nolting, S. P., Stockdale, G. D. & Thompson, G. D., Summary of the performance of spinosad against insect pests of crops. *Proc. ESA Annual Meeting, Dallas*, 13–17 December 1994.
3. Thompson, G. D., Busacca, J. D., Jantz, O. K. & Kirst, H. A., Spinosyns: an overview of new natural insect management systems. *Proc. ESA Annual Meeting, Dallas*, 13–17 December 1994.
4. Schooner, J. R. & Larson, L. L., Laboratory activity of spinosad on non-target beneficial arthropods. *Arthropod Management Tests*, **20** (1995) 357.
5. Fimiani, P., Mediterranean Region. In *World Crop Pests. Fruit Flies*, ed. A. S. Robinson & A. G. Hooper. Elsevier, Amsterdam, 1989, Vol. 3A, pp. 37–47.
6. Domínguez, F., *Plagas y enfermedades de las plantas cultivadas*. Dossat, Madrid, 1989, 821 pp. (in Spanish).
7. Mitchell, W. C. & Saul, S. H., Current control methods for the Mediterranean fruit fly *Ceratitis capitata*, and their application in USA. *Rev. Agric. Entomol.*, **78** (1990) 923–40.
8. Saul, S. H., Tsuda, D. & Wong, T. T. Y., Laboratory and field trials of soil applications of methoprene and other insecticides for control of the Mediterranean fruit fly. *J. Econ. Entomol.*, **76** (1983) 174–7.
9. Heather, N. W., Insecticidal dipping. In *World Crop Pests. Fruit Flies*, ed. A. S. Robinson & G. Hooper. Elsevier, Amsterdam, 1989, pp. 435–40.
10. Albajes, R. & Santiago-Alvarez, C., Efectos de la densidad larvaria y de la alimentación en la proporción de sexos de *Ceratitis capitata* (Dip., Trypetidae). *Anales INIA/Serie Agrícola*, **13** (1980) 175–82. (in Spanish).
11. Jacas, J. A. & Viñuela, E., Analysis of a laboratory method to test the effects of pesticides on adult females of *Opius concolor* (Hym, Braconidae), a parasitoid of the olive fruit fly, *Bactrocera oleae* (Dip., Tephritidae). *Bio-control Sci. Technol.*, **4** (1994) 147–54.
12. Viñuela, E., Influence of cold and carbon dioxide anaesthesia on the susceptibility of *Ceratitis capitata* adults to malathion. *Entomol. Exp. Appl.*, **32** (1982) 296–8.
13. Budia, F. & Viñuela, E., Effects of cyromazine fed continuously to adult *Ceratitis capitata* on mortality and reproduction. *J. Econ. Entomol.*, **89** (1996).
14. Potter, C., An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. *Ann. Appl. Biol.*, **39** (1952) 1–28.

15. Statgraphics, *User's Guide Statgraphics*. Graphic Software System STSC Inc., Rockville, MD, 1987.
16. LeOra Software, POLO-PC. *User's guide to probit or logit analysis*. LeOra Software Inc., Berkeley, CA, 1987.
17. Purcell, M. F., Stark, J. D. & Messing R. H., Insecticide effect on three tephritid fruit flies and associated braconid parasitoids in Hawaii. *J. Econ. Entomol.*, **87** (1994) 1455–62.
18. Schuster, D. J. & Taylor, J. L., Longevity and oviposition of adult *Liriomyza trifolii* (Dip., Agromyzidae) exposed to abamectin in the laboratory. *J. Econ. Entomol.*, **81** (1988) 106–9.
19. Lasota, J. A. & Dybas, R. A., Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Ann. Rev. Entomol.*, **36** (1991) 91–117.
20. Strong, L. & Brown, T. A., Avemectins in insect control and biology: a review. *Bull. Entomol. Res.*, **77** (1987) 357–89.
21. Albrecht, C. P. & Sherman, M., Lethal and sublethal effects of avermectin B₁ on three fruit fly species (Diptera: Tephritidae). *J. Econ. Entomol.*, **80** (1987) 344–7.
22. Grosscurt, A. C. & Jongsma, B., Mode of action and insecticidal properties of diflubenzuron. In *Chitin and benzoylphenyl ureas*, ed. J. E. Wright & A. Retnakaran. Dr Junk Publ., The Netherlands, 1987, pp. 75–99.
23. Viñuela, E., Budia, F., Jacas, J. A., Adán, A., Marco, V. & Del Estal, P., Differential larval age susceptibility of the medfly, *Ceratitis capitata* to cyromazine. *J. App. Entomol.*, **115** (1993) 355–62.
24. Budia, F., Efectos del RCI ciromacina sobre la mosca mediterránea de la fruta *Ceratitis capitata* Wied. (Dip., Tephritidae). *Tesis Doct. UPM, Madrid*, 1992, 234 pp. (in Spanish).
25. Stark, J. D., Vargas, R. I., Messing, R. H. & Purcell, M., Effects of cyromazine and diazinon on three economically important Hawaiian tephritid fruit flies (Diptera: Tephritidae) and their endoparasitoids (Hymenoptera: Braconidae). *J. Econ. Entomol.*, **85** (1992) 1687–94.
26. Abbott, W. S., A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18** (1925) 265–7.